

Supplemental Materials for

The landscape of peripheral blood RNA modifications and its clinical implications for diagnosis of hepatocellular carcinoma

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Figure S1

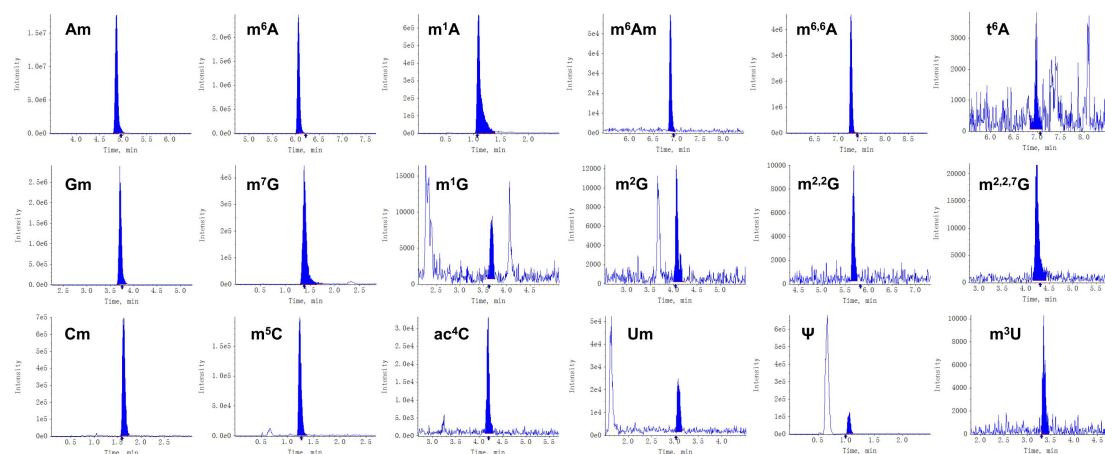


Figure S1. Representative MRM chromatograms of the detectable RNA modifications in one peripheral blood sample

Figure S2

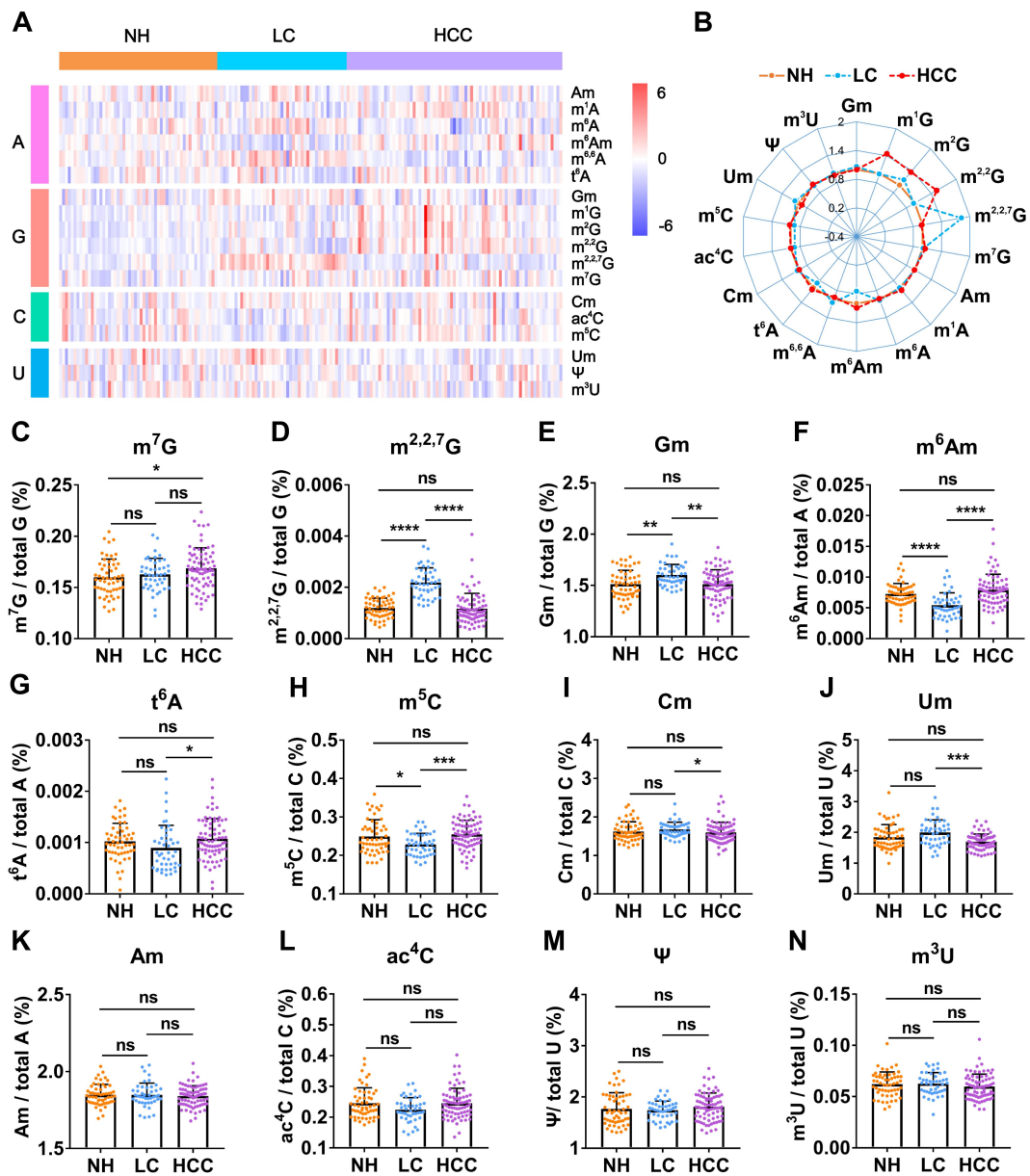


Figure S2. Abundance of multiple RNA modifications in peripheral blood among NH individuals, LC patients, and HCC patients

(A) Heatmap for the relative abundances of detectable peripheral blood RNA modifications in the NH, LC, and HCC groups;

(B) Changes in the average relative abundances of detectable peripheral blood RNA modifications in the HCC and LC groups, compared with the NH group;

(C–L) The relative abundances of m⁷G (C), m^{2,2,7}G (D), Gm (E), m⁶Am (F), t⁶A(G), m⁵C (H), Cm (I), Um (J), Am (K), ac⁴C (L), Ψ (M), and m³U (N) in the NH, LC, and HCC groups.

P values were adjusted using the Benjamini-Hochberg FDR method (*Q* = 0.05). An adjusted *p* value < 0.05 was considered statistically significant. **p* < 0.05; ***p* < 0.01;

****p* < 0.001; *****p* < 0.0001; ns, not significant.

Figure S3

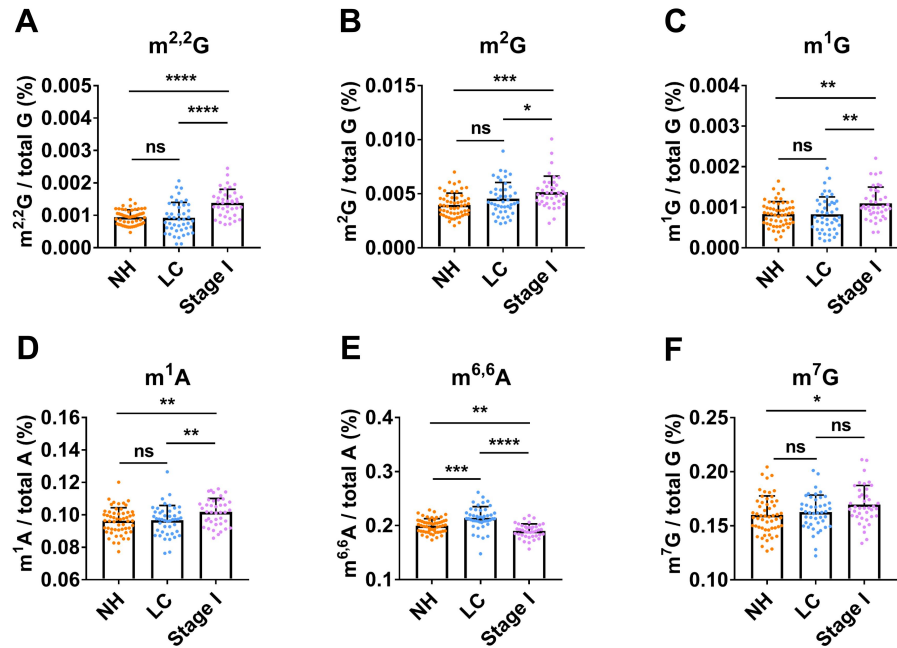


Figure S3. Abundance of multiple RNA modifications in peripheral blood among NH individuals, LC patients, and stage I HCC patients

The relative abundances of $m^{2,2}G$ (A), m^2G (B), m^1G (C), m^1A (D), $m^{6,6}A$ (E), and m^7G (F) in peripheral blood of NH individuals ($n = 55$), LC patients ($n = 45$) and stage I HCC patients ($n = 40$).

P values were adjusted using the Benjamini-Hochberg FDR method ($Q = 0.05$). An adjusted p value < 0.05 was considered statistically significant. * $p < 0.05$; ** $p < 0.01$;

*** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

Figure S4

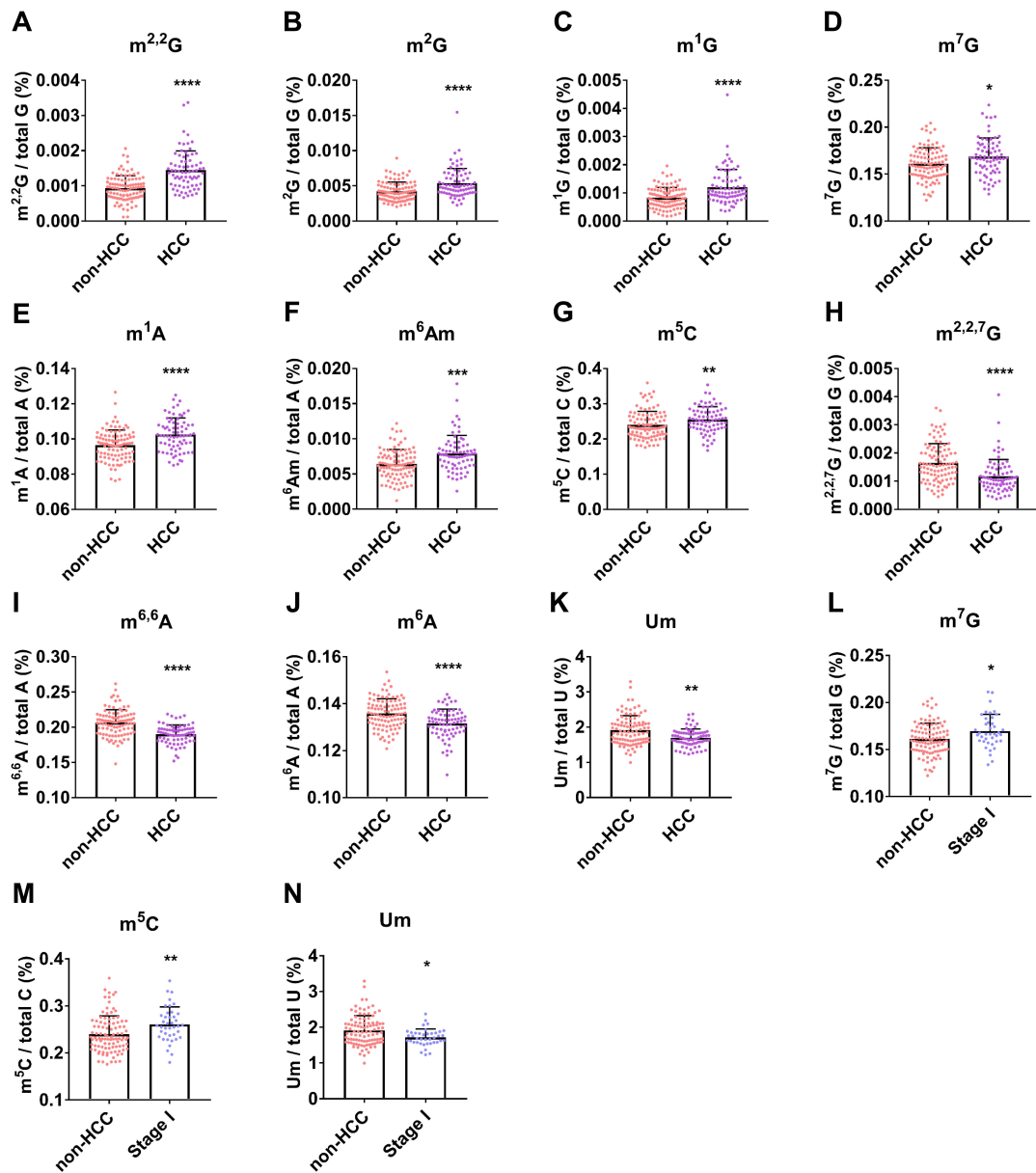


Figure S4. Abundance of multiple RNA modifications in peripheral blood between non-HCC individuals and HCC patients

(A–K) The relative abundances of $m^{2,2}G$ (A), m^2G (B), m^1G (C), m^7G (D), m^1A (E), m^6Am (F), m^5C (G), $m^{2,2,7}G$ (H), $m^{6,6}A$ (I), m^6A (J), and Um (K) in the non-HCC (NH and LC) and HCC groups;

(L–M) The relative abundances of m^7G (L), m^5C (M), and Um (N) in the non-HCC

and stage I HCC groups.

P values were adjusted using the Benjamini-Hochberg FDR method ($Q = 0.05$). An

adjusted *p* value < 0.05 was considered statistically significant. * $p < 0.05$; ** $p < 0.01$;

*** $p < 0.001$; **** $p < 0.0001$.

Figure S5

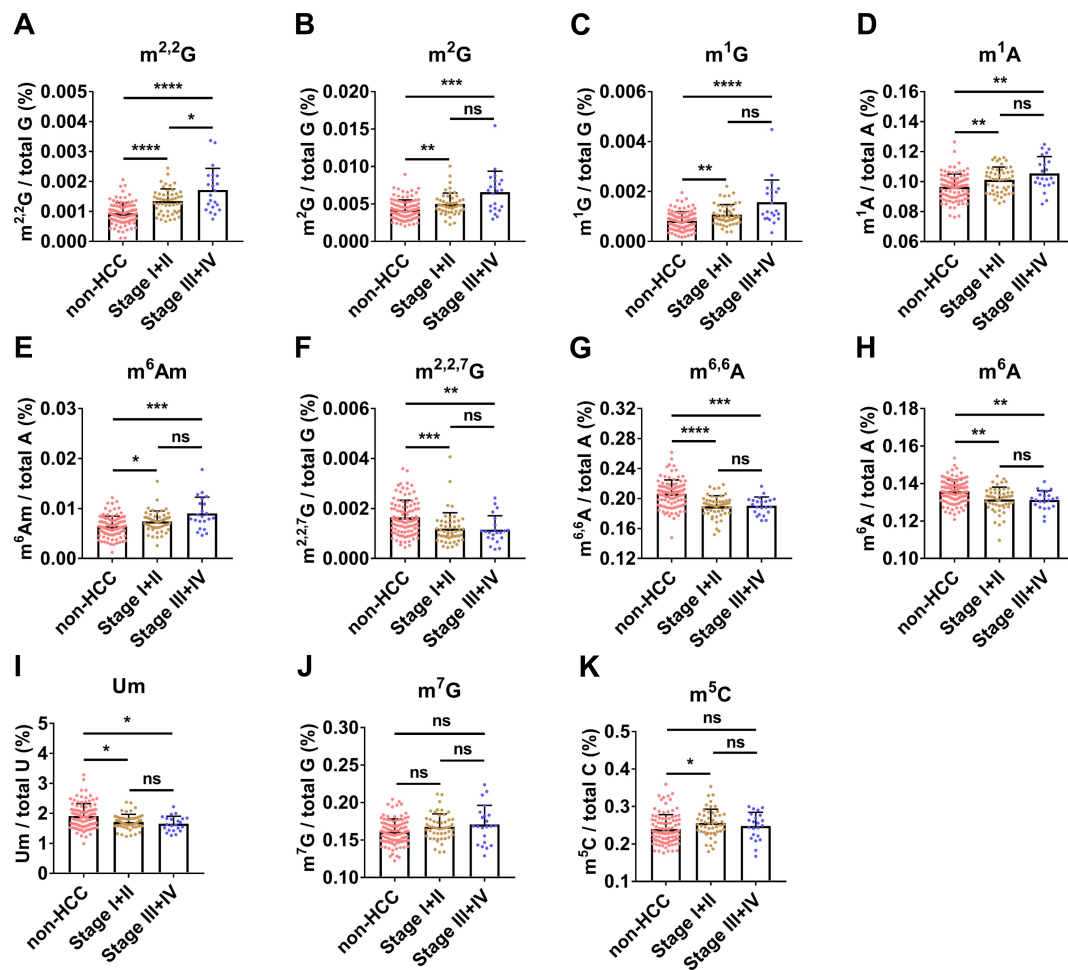


Figure S5. Abundance of multiple RNA modifications in peripheral blood among non-HCC individuals and HCC patients with different stages

The relative abundances of m^{2,2}G (A), m²G (B), m¹G (C), m¹A (D), m⁶Am (E), m^{2,2,7}G (F), m^{6,6}A (G), m⁶A (H), Um (I), m⁷G (J), and m⁵C (K) in peripheral blood of non-HCC individuals, HCC patients with early/intermediate-stage (stages I-II) and advanced/terminal-stage (stages III-IV). Of the 75 HCC patients, 40 had stage I, 10 had stage II, 19 had stage III, and 3 had stage IV, based on the China Liver Cancer Staging (CNLC) system. Data on stage were unavailable for the remaining 3 patients. *P* values were adjusted using the Benjamini-Hochberg FDR method (*Q* = 0.05). An

adjusted p value < 0.05 was considered statistically significant. * $p < 0.05$; ** $p < 0.01$;

*** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

Figure S6

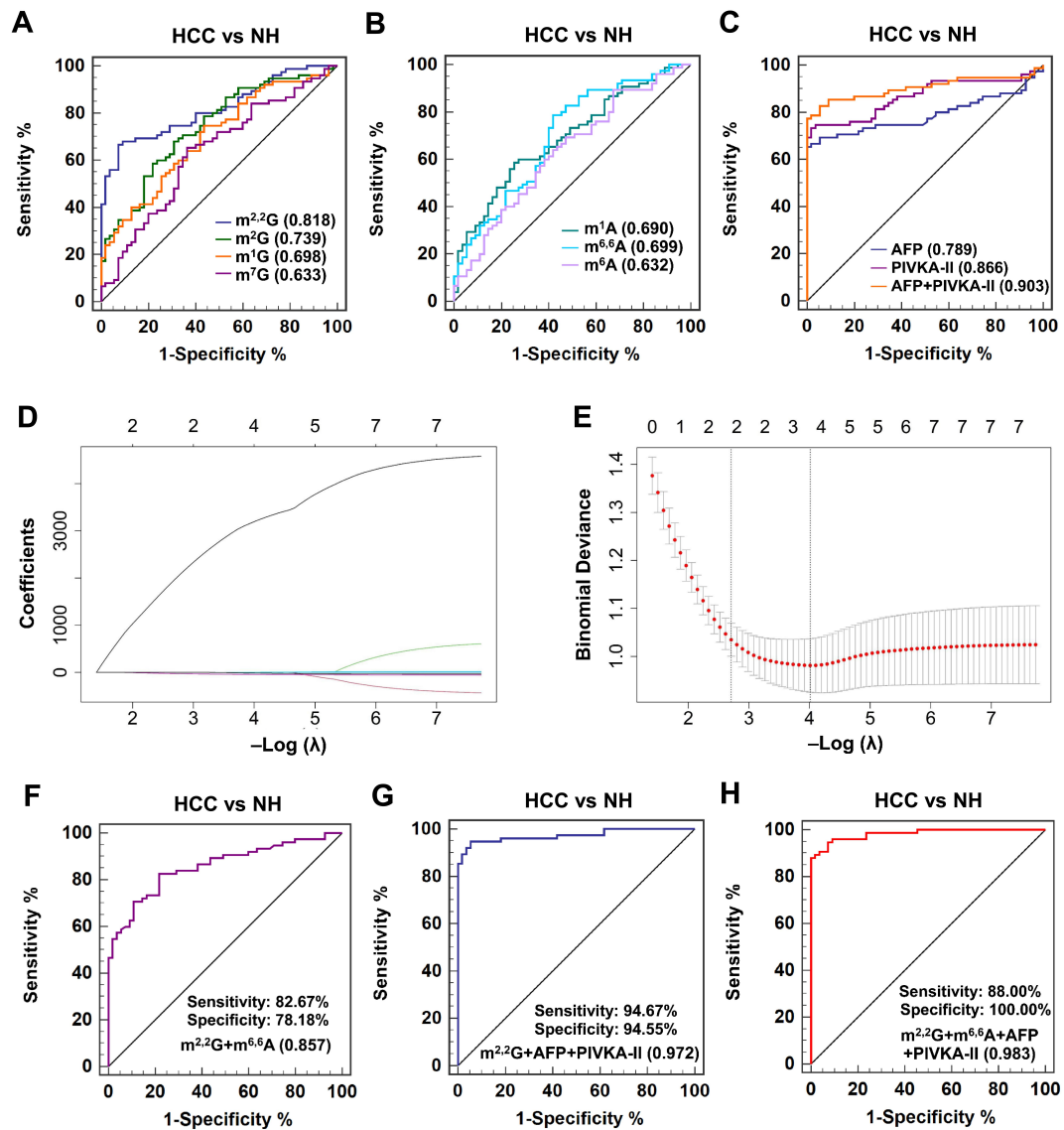


Figure S6. Diagnostic performance of peripheral blood RNA modifications in HCC

- (A) The ROC curves for $m^{2,2}G$, m^2G , m^1G , and m^7G individually to discriminate between HCC patients and NH individuals;
- (B) The ROC curves for m^1A , $m^{6,6}A$, and m^6A individually to discriminate between HCC patients and NH individuals;
- (C) The ROC curves for AFP, and PIVKA-II individually, a model combining AFP,

and PIVKA-II to discriminate between HCC patients and NH individuals;

(D) LASSO coefficient profiles of 7 RNA modifications with differential abundance

between the NH and HCC groups;

(E) 10-fold cross-validation for selection of the optimal λ value among 7 RNA

modifications;

(F) The ROC curve for a model combining m^{2,2}G and m^{6,6}A to discriminate between

HCC patients and NH individuals;

(G) The ROC curve for a model combining m^{2,2}G, AFP, and PIVKA-II to

discriminate between HCC patients and NH individuals;

(H) The ROC curve for a model combining m^{2,2}G, m^{6,6}A, AFP, and PIVKA-II to

discriminate between HCC patients and NH individuals.

Figure S7

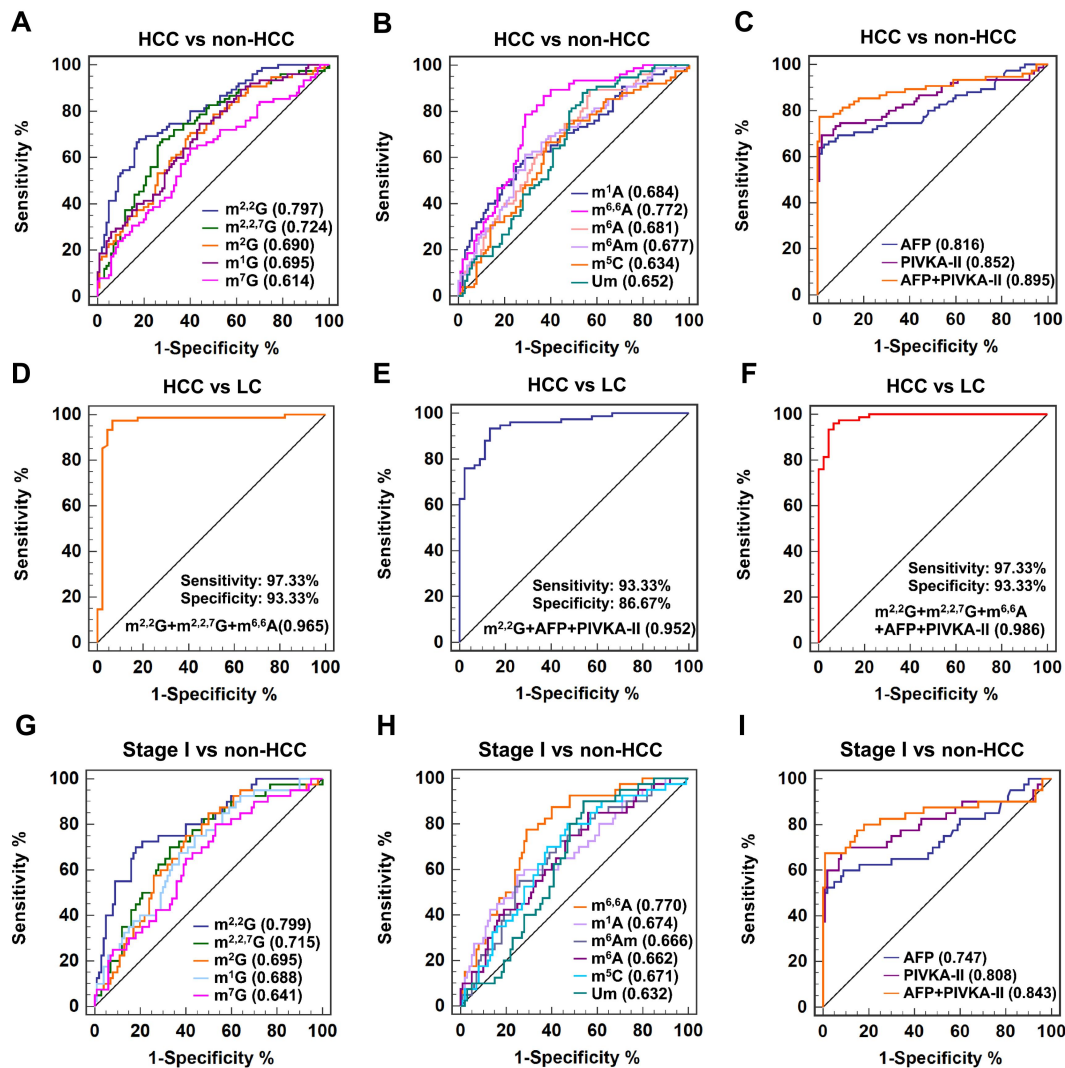


Figure S7. Diagnostic performance of peripheral blood RNA modifications in HCC

- (A) The ROC curves for $m^{2,2}G$, $m^{2,2,7}G$, m^2G , m^1G , and m^7G individually to discriminate between HCC patients and non-HCC individuals;
- (B) The ROC curves for m^1A , $m^{6,6}A$, m^6A , m^6Am , m^5C , and Um individually to discriminate between HCC patients and non-HCC individuals;
- (C) The ROC curves for AFP, and PIVKA-II individually, a model combining AFP and PIVKA-II to discriminate between HCC patients and non-HCC individuals;

- (D) The ROC curve for a model combining $m^{2,2}G$, $m^{2,2,7}G$, and $m^{6,6}A$ to discriminate between HCC patients and LC patients;
- (E) The ROC curve for a model combining $m^{2,2}G$, AFP, and PIVKA-II to discriminate between HCC patients and LC patients;
- (F) The ROC curve for a model combining $m^{2,2}G$, $m^{2,2,7}G$, $m^{6,6}A$, AFP, and PIVKA-II to discriminate between HCC patients and LC patients;
- (G) The ROC curves for $m^{2,2}G$, $m^{2,2,7}G$, m^2G , m^1G , and m^7G individually to discriminate between stage I HCC patients and non-HCC individuals;
- (H) The ROC curves for $m^{6,6}A$, m^1A , m^6Am , m^6A , m^5C , and Um individually to discriminate between stage I HCC patients and non-HCC individuals;
- (I) The ROC curves for AFP, and PIVKA-II individually, a model combining AFP, and PIVKA-II to discriminate between stage I HCC patients and non-HCC individuals.

A non-HCC

B non-HCC

C Stage I HCC

D Stage I HCC

E non-HCC

F Stage I

G non-HCC

H Stage I

I non-HCC

J Stage I

K non-HCC

L Stage I

M Stage I

N Stage I

O Stage I

P Stage I

Q Stage I

R Stage I

(A) Pearson correlation-based crosstalk network of peripheral blood RNA modification abundances was visualized using chord diagram for the non-HCC group;

- (B) Heatmap showed Pearson correlations among peripheral blood RNA modification abundances in the non-HCC group;
- (C) Pearson correlation-based crosstalk network of peripheral blood RNA modification abundances was visualized using chord diagram for the stage I HCC group;
- (D) Heatmap showed Pearson correlations among peripheral blood RNA modification abundances in the stage I HCC group;
- (E–F) Pearson correlations between m^2G abundance and that of $m^{2,2}G$ in peripheral blood were shown for the non-HCC (E), and stage I HCC (F) groups;
- (G–H) Pearson correlations between m^1G abundance and that of $m^{2,2}G$ in peripheral blood were shown for the non-HCC (G), and stage I HCC (H) groups;
- (I–J) Pearson correlations between m^1G abundance and that of m^2G in peripheral blood were shown for the non-HCC (I), and stage I HCC (J) groups;
- (K–L) Pearson correlations between $m^{6,6}A$ abundance and that of m^6A in peripheral blood were shown for the non-HCC (K), and stage I HCC (L) groups;
- (M) Pearson correlation between m^7G abundance and that of $m^{2,2}G$ in peripheral blood was shown for the stage I HCC group;
- (N–O) Pearson correlations between $m^{2,2,7}G$ abundance and that of m^2G (N), and m^1G (O) in peripheral blood were shown for the stage I HCC group;
- (P) Pearson correlation between m^6A abundance and that of m^1A in peripheral blood was shown for the stage I HCC group;
- (Q) Pearson correlation between m^1A abundance and that of m^2G in peripheral blood

was shown for the stage I HCC group;

(R) Pearson correlation between Um abundance and that of m⁶A in peripheral blood

was shown for the stage I HCC group.

Pearson correlation coefficients were computed using GraphPad Prism.

Figure S9

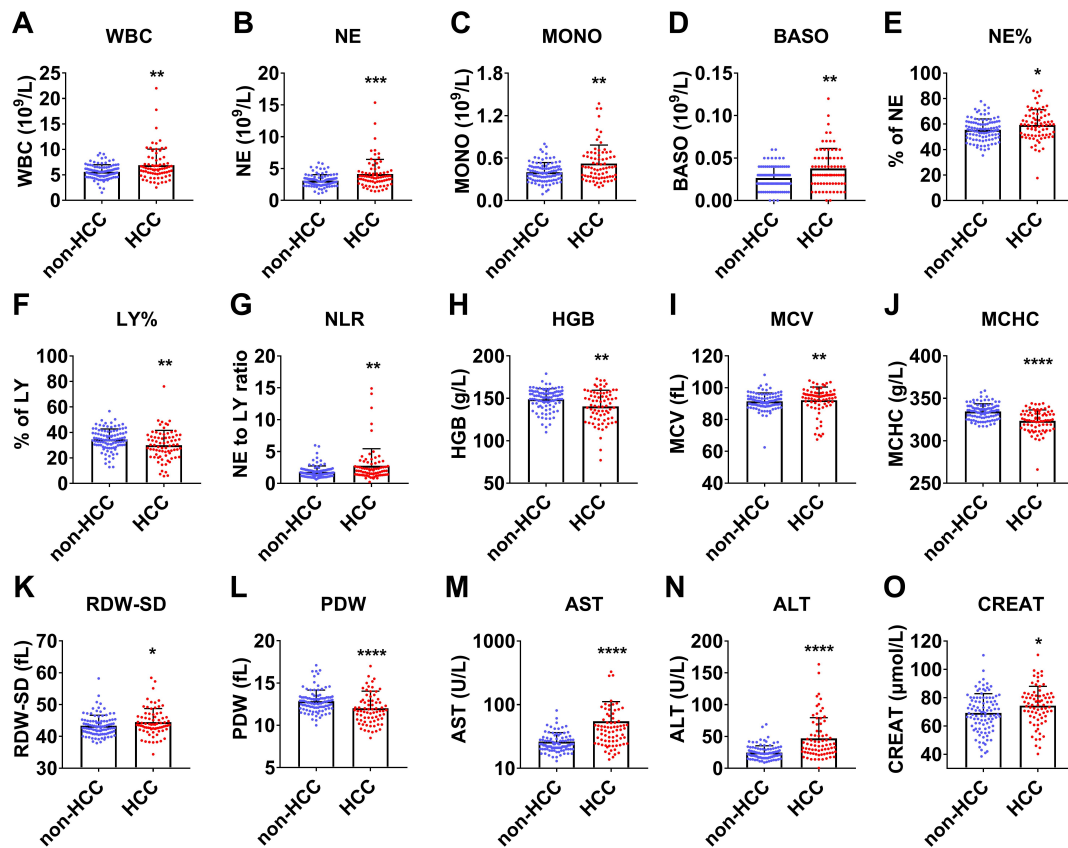


Figure S9. Alterations in clinical laboratory parameters between non-HCC individuals and HCC patients

(A–G) Alterations in white blood cell parameters including WBC (A), NE (B), MONO (C), BASO (D), NE% (E), LY% (F), and NLR (G) between non-HCC individuals and HCC patients;

(H–K) Alterations in red blood cell parameters including HGB (H), MCV (I), MCHC (J), and RDW-SD (K) between non-HCC individuals and HCC patients;

(L) Alterations in platelet parameter PDW between non-HCC individuals and HCC patients;

(M–O) Alterations in biochemical parameters including AST (M), ALT (N), and

CREAT (O) between non-HCC individuals and HCC patients.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure S10

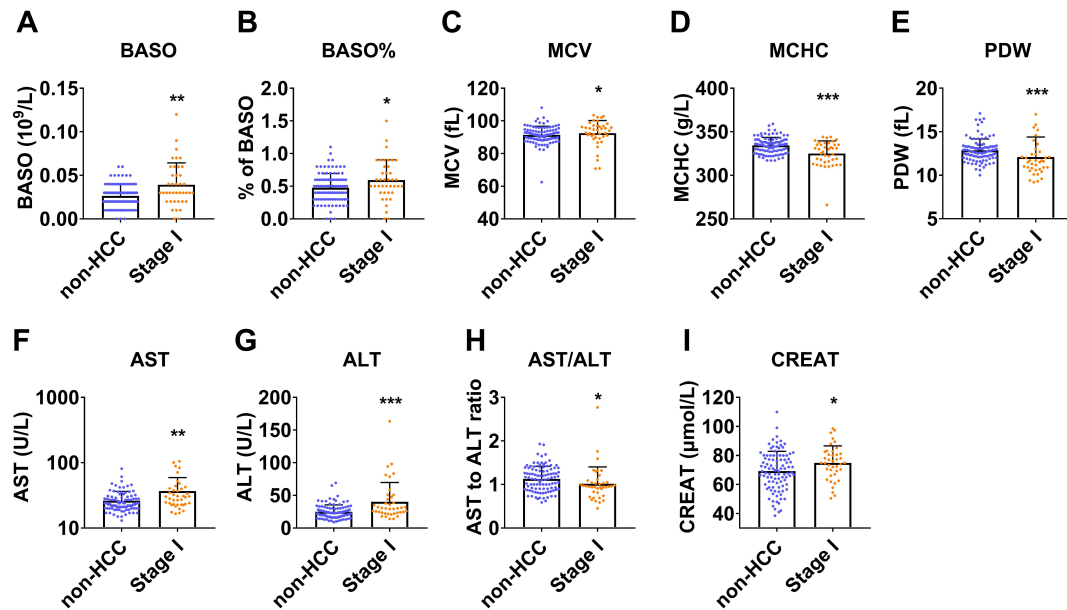


Figure S10. Alterations in clinical laboratory parameters between non-HCC individuals and stage I HCC patients

(A–B) Alterations in white blood cell parameters including BASO (A), and BASO%

(B) between non-HCC individuals and stage I HCC patients;

(C–D) Alterations in red blood cell parameters including MCV (C), and MCHC (D)

between non-HCC individuals and stage I HCC patients;

(E) Alterations in platelet parameter PDW between non-HCC individuals and stage I

HCC patients;

(F–I) Alterations in biochemical parameters including AST (F), ALT (G), AST/ALT

(H), and CREAT (I) between non-HCC individuals and stage I HCC patients.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure S11

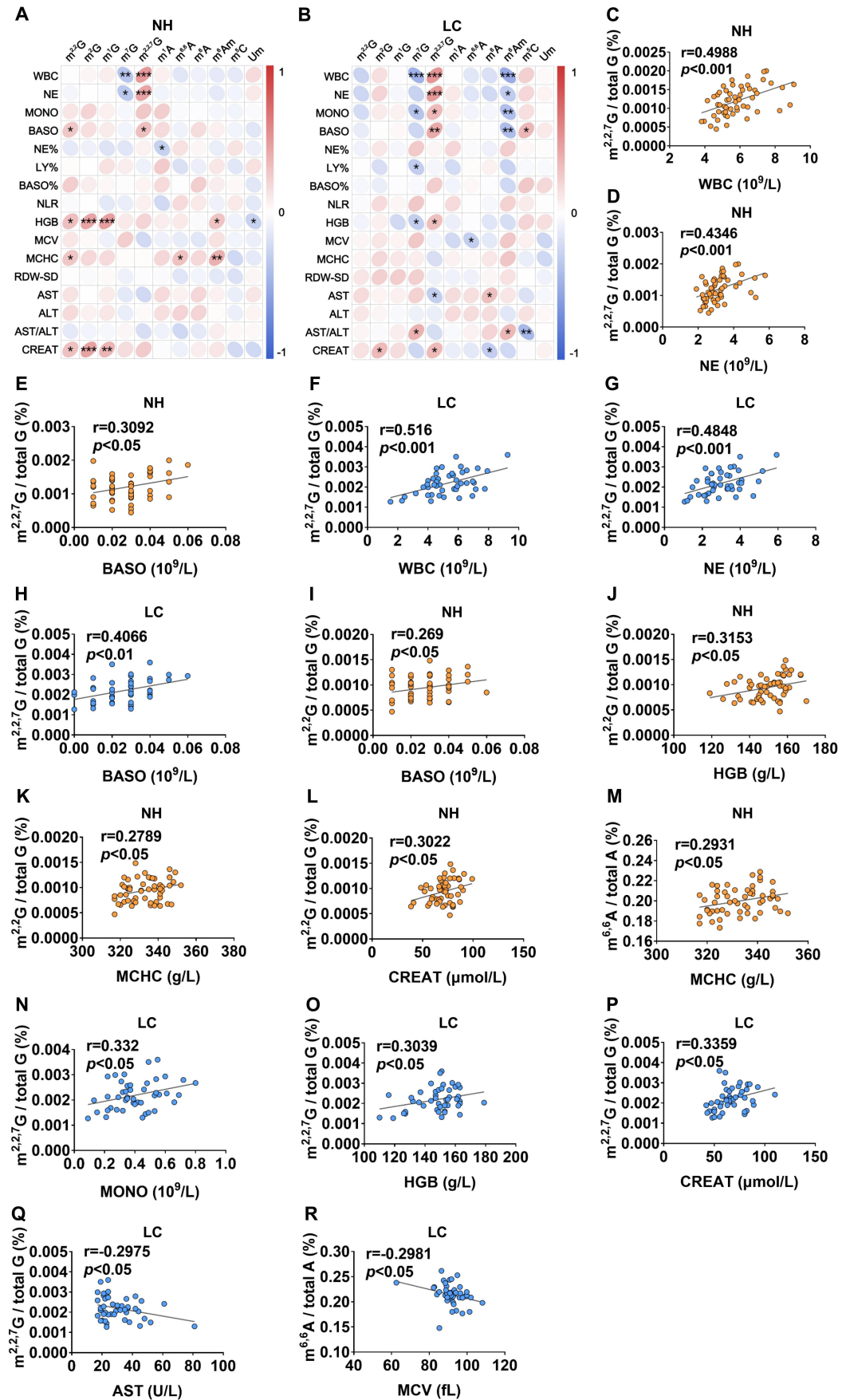


Figure S11. Correlations between peripheral blood RNA modifications and laboratory parameters in the NH and LC groups

(A–B) Heatmaps showed Pearson correlations between peripheral blood RNA modification abundances and laboratory parameters in the NH (A) and LC (B) groups;

(C–E) Pearson correlations between m^{2,2,7}G abundance in peripheral blood and WBC (C), NE (D), and BASO (E) were shown for the NH group;

(F–H) Pearson correlations between m^{2,2,7}G abundance in peripheral blood and WBC (F), NE (G), and BASO (H) were shown for the LC group;

(I–L) Pearson correlations between m^{2,2}G abundance in peripheral blood and BASO (I), HGB (J), MCHC (K), and CREAT (L) were shown for the NH group;

(M) Pearson correlation between m^{6,6}A abundance in peripheral blood and MCHC was shown for the NH group;

(N–Q) Pearson correlations between m^{2,2,7}G abundance in peripheral blood and MONO (N), HGB (O), CREAT (P), and AST (Q) were shown for the LC group;

(R) Pearson correlation between m^{6,6}A abundance in peripheral blood and MCV was shown for the LC group.

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Pearson correlation coefficients were computed using GraphPad Prism.

Figure S12

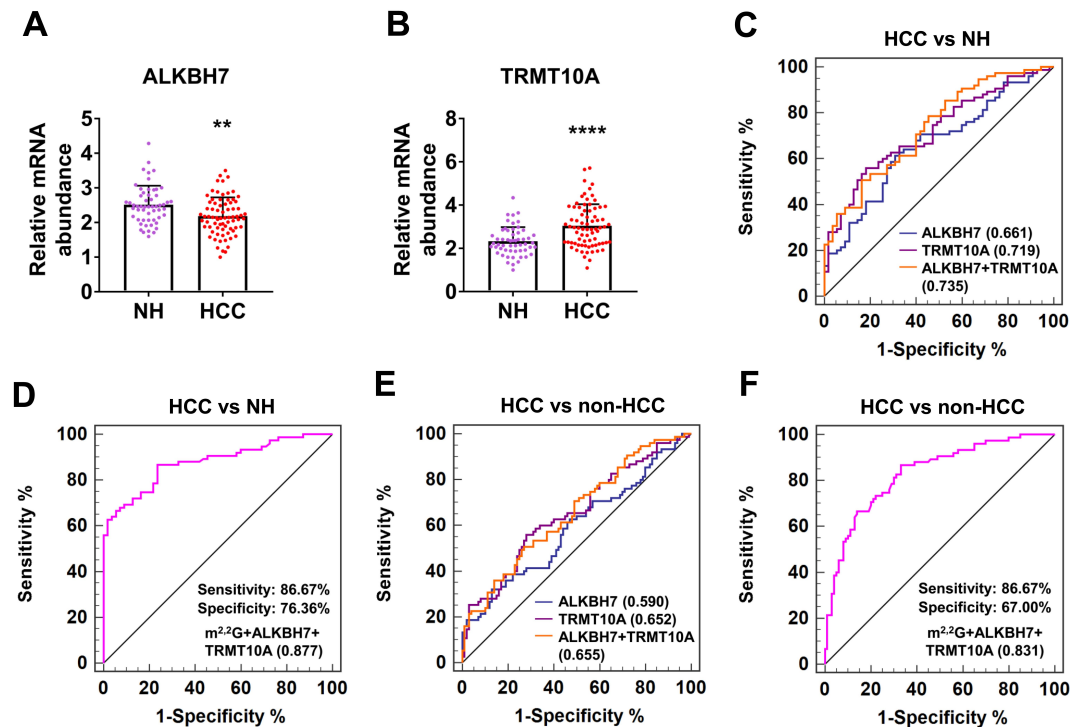


Figure S12. Altered abundances and diagnostic performance of ALKBH7 and TRMT10A in HCC

- (A–B) The relative mRNA abundances of ALKBH7 (A), and TRMT10A (B) in peripheral blood of NH individuals (n = 55) and HCC patients (n = 75) were measured by RT- qPCR analysis;
- (C) The ROC curves for ALKBH7, and TRMT10A individually, a model combining ALKBH7 and TRMT10A to discriminate between HCC patients and NH individuals;
- (D) The ROC curve for a model combining $m^{2,2}G$, ALKBH7, and TRMT10A to discriminate between HCC patients and NH individuals;
- (E) The ROC curves for ALKBH7, and TRMT10A individually, a model combining ALKBH7 and TRMT10A to discriminate between HCC patients and non-HCC

individuals;

(F) The ROC curve for a model combining m^{2,2}G, ALKBH7, and TRMT10A to discriminate between HCC patients and non-HCC individuals.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure S13

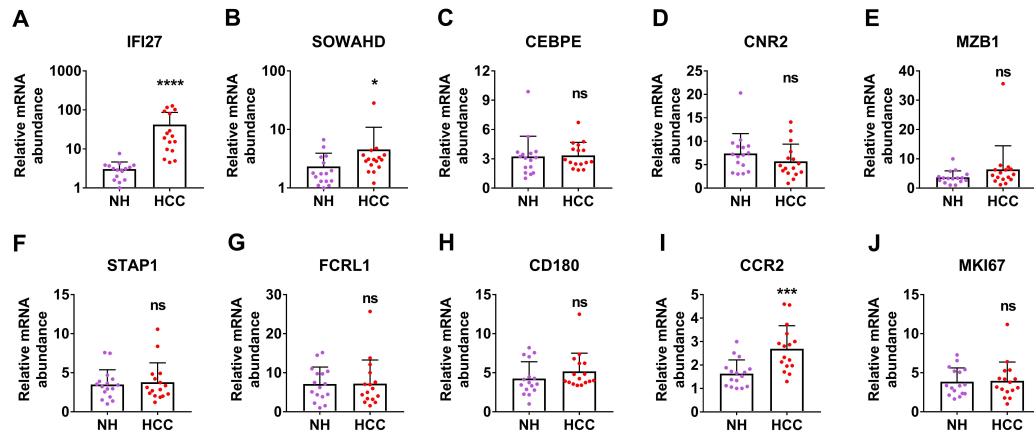


Figure S13. RT-qPCR validation of the top 10 upregulated genes identified from RNA-seq data in the HCC group.

The relative mRNA abundances of IFI27 (A), SOWAHD (B), CEBPE (C), CNR2 (D), MZB1 (E), STAP1 (F), FCRL1 (G), CD180 (H), CCR2 (I), and MKI67 (J) in peripheral blood of NH individuals and HCC patients (n = 16, per group) were investigated by RT-qPCR analysis.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

Figure S14

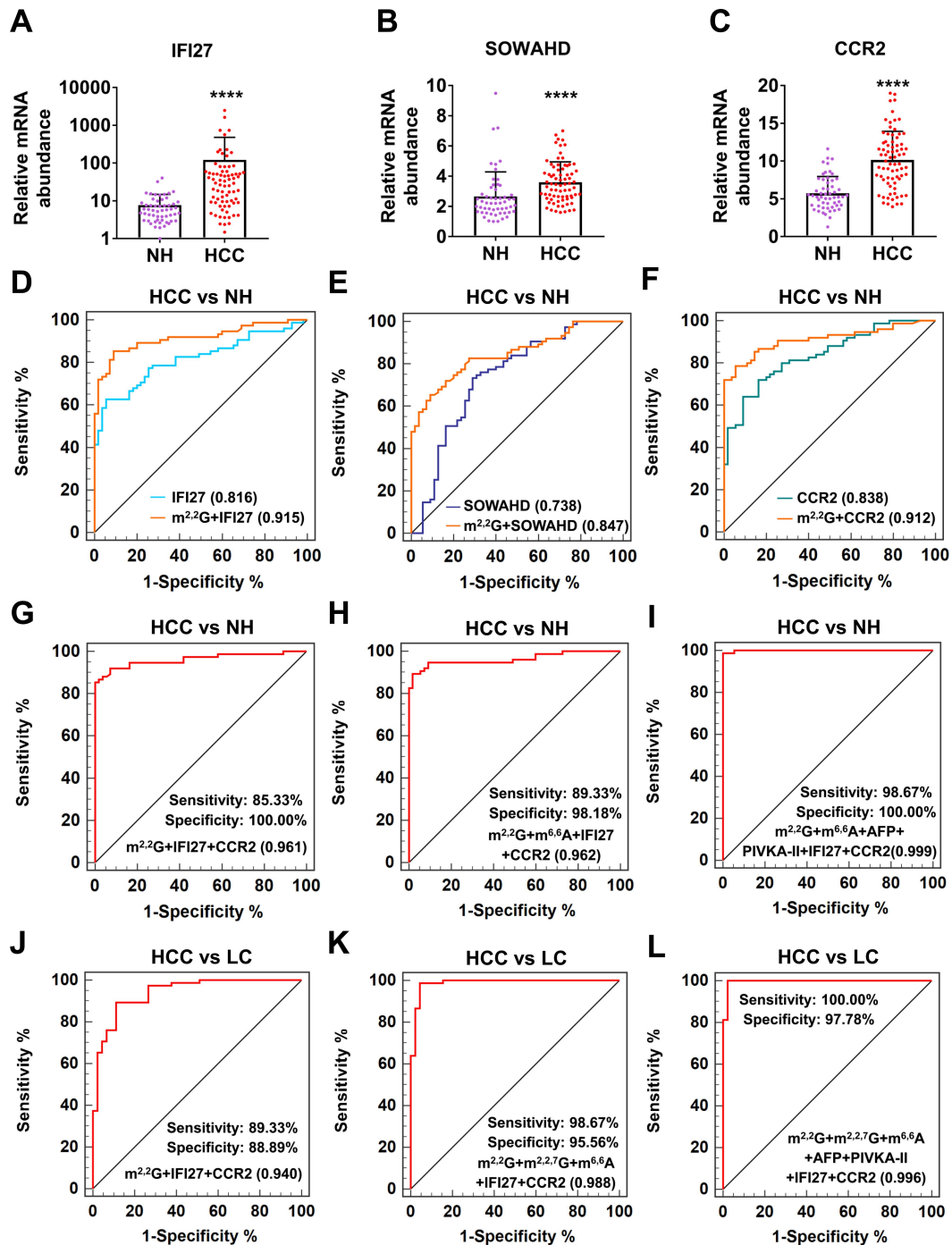


Figure S14. Altered abundances and diagnostic performance of IFI27, SOWAHD, and CCR2 in HCC.

(A–C) The relative mRNA abundances of IFI27 (A), SOWAHD (B), and CCR2 (C)

in peripheral blood of NH individuals ($n = 55$) and HCC patients ($n = 75$) were

measured by RT-qPCR analysis;

- (D) The ROC curves for IFI27 individually, a model combining m^{2,2}G and IFI27 to discriminate between HCC patients and NH individuals;
- (E) The ROC curves for SOWAHD individually, a model combining m^{2,2}G and SOWAHD to discriminate between HCC patients and NH individuals;
- (F) The ROC curves for CCR2 individually, a model combining m^{2,2}G and CCR2 to discriminate between HCC patients and NH individuals;
- (G) The ROC curve for a model combining m^{2,2}G, IFI27, and CCR2 to discriminate between HCC patients and NH individuals;
- (H) The ROC curve for a model combining m^{2,2}G, m^{6,6}A, IFI27, and CCR2 to discriminate between HCC patients and NH individuals;
- (I) The ROC curve for a model combining m^{2,2}G, m^{6,6}A, AFP, PIVKA-II, IFI27, and CCR2 to discriminate between HCC patients and NH individuals;
- (J) The ROC curve for a model combining m^{2,2}G, IFI27, and CCR2 to discriminate between HCC patients and LC patients;
- (K) The ROC curve for a model combining m^{2,2}G, m^{2,2,7}G, m^{6,6}A, IFI27, and CCR2 to discriminate between HCC patients and LC patients;
- (L) The ROC curve for a model combining m^{2,2}G, m^{2,2,7}G, m^{6,6}A, AFP, PIVKA-II, IFI27, and CCR2 to discriminate between HCC patients and LC patients.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure S15

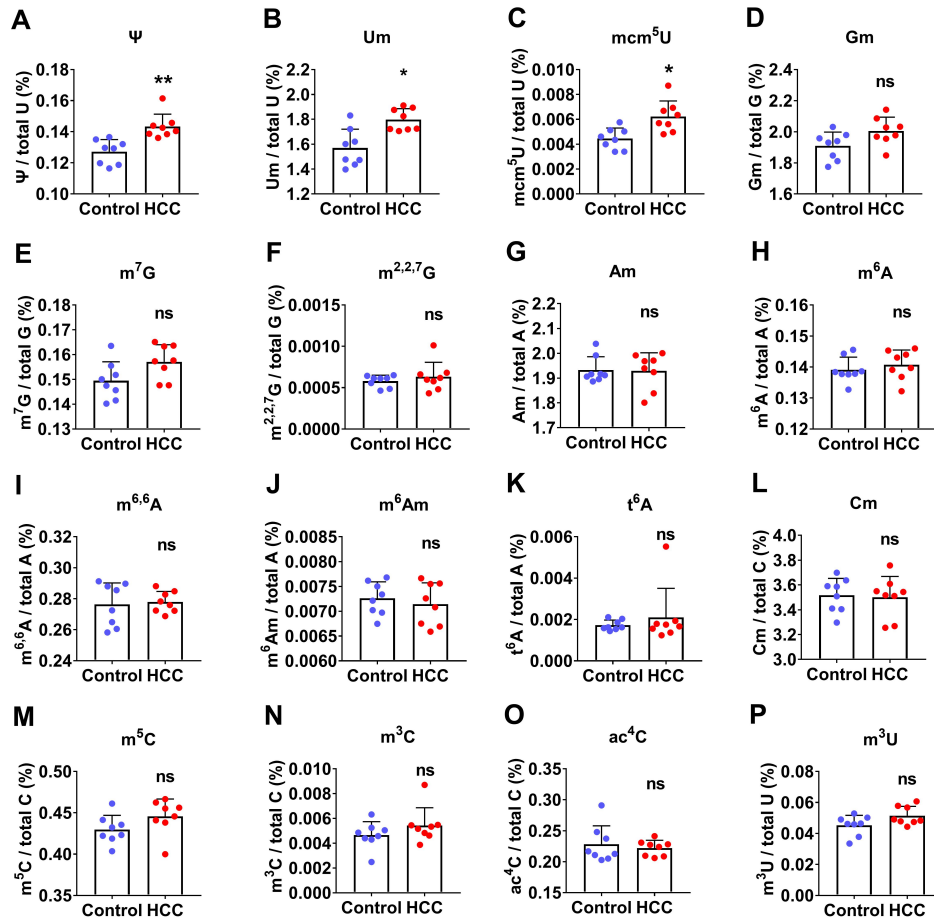


Figure S15. Abundance of multiple RNA modifications in control mice and the syngeneic HCC mouse model

The relative abundances of Ψ (A), Um (B), mcm⁵U (C), Gm (D), m⁷G (E), m^{2,2,7}G (F), Am (G), m⁶A (H), m^{6,6}A (I), m⁶Am (J), t⁶A (K), Cm (L), m⁵C (M), m³C (N), ac⁴C (O), and m³U (P) in C57BL/6 mice blood of the control and HCC groups (n = 8, per group).

P values were adjusted using the Benjamini-Hochberg FDR method (*Q* = 0.05). An adjusted *p* value < 0.05 was considered statistically significant. * *p* < 0.05; ** *p* < 0.01;

*** *p* < 0.001; **** *p* < 0.0001; ns, not significant.

Figure S16

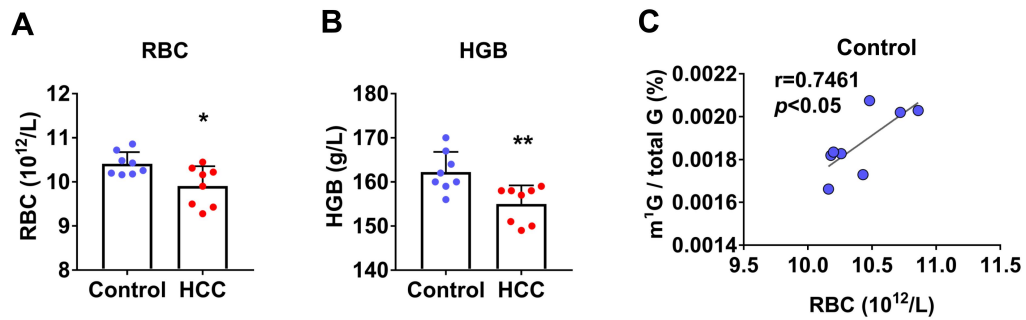


Figure S16. Alterations in laboratory parameters in the syngeneic HCC mouse model and their correlation with blood RNA modifications

(A–B) Alterations in RBC (A) and HGB (B) of C57BL/6 mice between the control and HCC groups (n = 8, per group);

(M) Pearson correlation between blood m¹G abundance and RBC was shown for the control group.

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Pearson correlation coefficients were computed using GraphPad Prism.

Figure S17

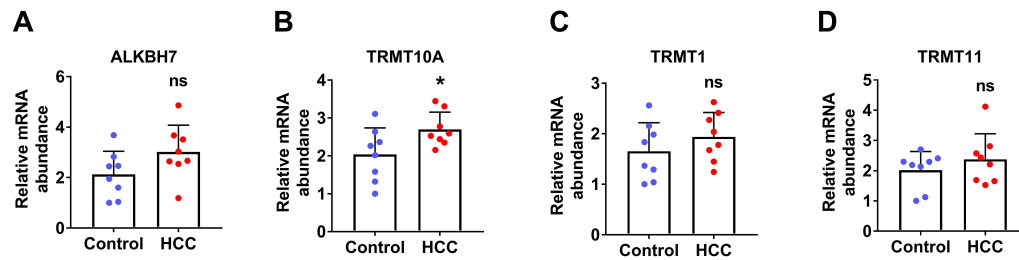


Figure S17. Abundances of methyltransferases and demethylases for specific RNA modifications in control mice and the syngeneic HCC mouse model

The relative mRNA abundances of ALKBH7 (A), TRMT10A (B), TRMT1 (C), and TRMT11 (D) in C57BL/6 mice blood of the control and HCC groups (n = 8, per group) were checked by RT-qPCR analysis.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

Figure S18

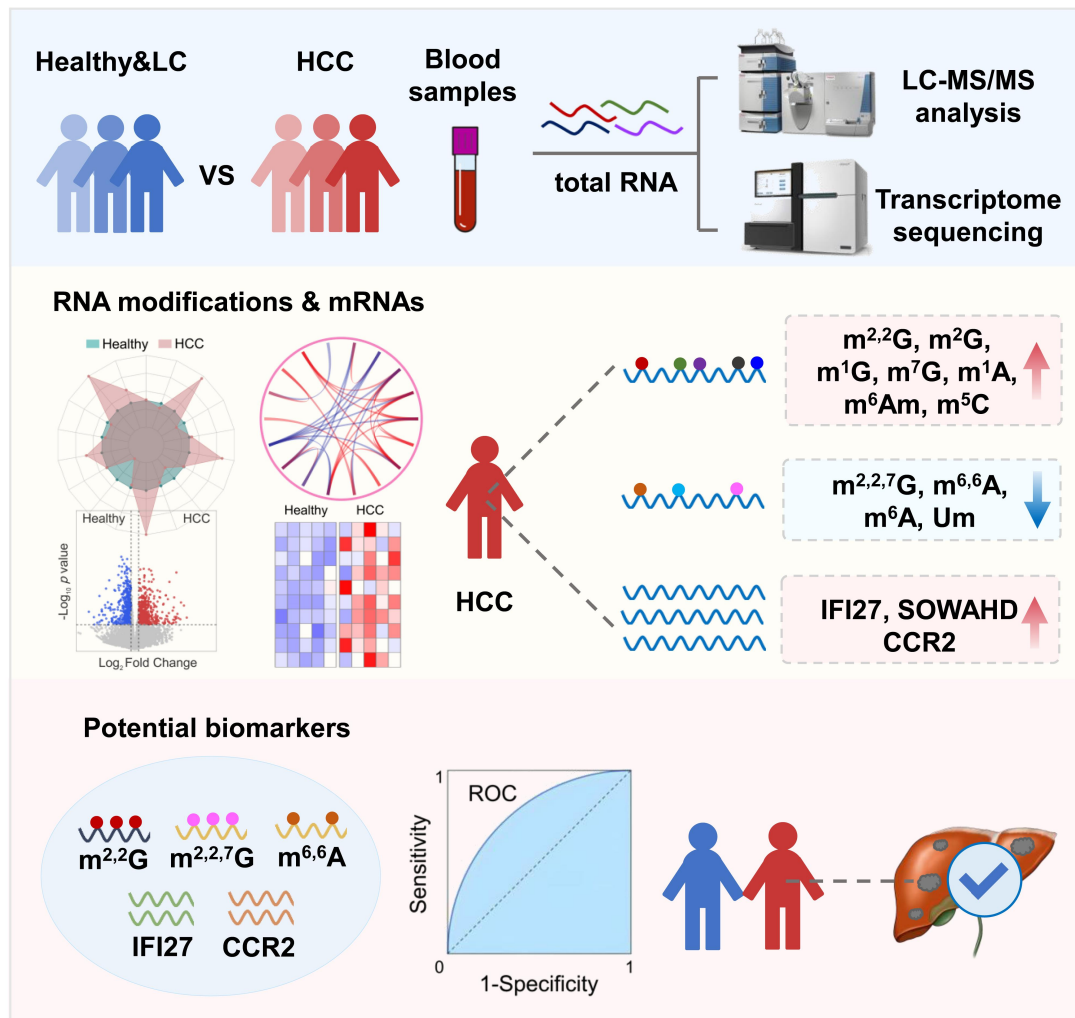


Figure S18. The graphical abstract of our study

Supplementary Tables

Supplementary Table S1. Baseline clinical characteristics.

Please see separated Excel spreadsheet file.

Supplementary Table S2. The 55 types of RNA modifications were measured by LC-MS/MS method.

Please see separated Excel spreadsheet file.

Supplementary Table S3. The optimized MRM parameters for analysis of canonical, modified nucleosides by LC-MS/MS method.

Please see separated Excel spreadsheet file.

Supplementary Table S4. Quantitative real-time PCR primers used in this study.

Please see separated Excel spreadsheet file.

Supplementary Table S5. LASSO and multivariate logistic regression analyses for RNA modifications.

Please see separated Excel spreadsheet file.

Supplementary Table S6. Diagnostic performance of individual and combined markers for discriminating HCC patients from NH individuals.

Please see separated Excel spreadsheet file.

Supplementary Table S7. Diagnostic performance of individual and combined markers for discriminating HCC patients from non-HCC individuals.

Please see separated Excel spreadsheet file.

Supplementary Table S8. Diagnostic performance of combined markers for discriminating HCC patients from LC patients.

Please see separated Excel spreadsheet file.

Supplementary Table S9. Diagnostic performance of individual and combined markers for discriminating stage I HCC patients from non-HCC individuals.

Please see separated Excel spreadsheet file.

Supplementary Table S10. Identified methyltransferases and demethylases for specific RNA modifications.

Please see separated Excel spreadsheet file.

Supplementary Table S11. Multivariate logistic regression analysis for mRNA markers.

Please see separated Excel spreadsheet file.

Supplementary Table S12. Diagnostic performance of individual and combined markers for discriminating HCC patients from NH individuals.

Please see separated Excel spreadsheet file.

Supplementary Table S13. Diagnostic performance of individual and combined markers for discriminating HCC patients from non-HCC individuals.

Please see separated Excel spreadsheet file.

Supplementary Table S14. Diagnostic performance of combined markers for discriminating HCC patients from LC patients.

Please see separated Excel spreadsheet file.

Supplementary Table S15. Diagnostic performance of individual and combined markers for discriminating stage I HCC patients from non-HCC individuals.

Please see separated Excel spreadsheet file.

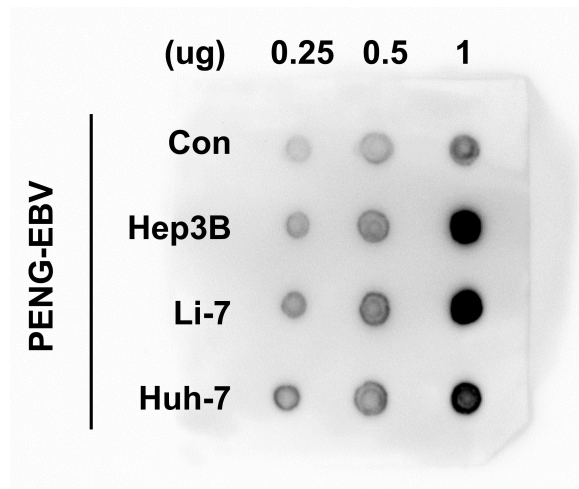
Supplementary Table S16. Internal validation for multivariate diagnostic models.

Please see separated Excel spreadsheet file.

Supplementary Table S17. Multivariable-adjusted associations of key RNA modifications with HCC after adjustment for clinical confounders.

Please see separated Excel spreadsheet file.

Original Gels/Blots images



Original cellular m^{2,2}G abundance for Fig. 7B



Original cellular ALKBH7 and β-tubulin expression for Fig. 7C